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6/10/02
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DECLARATION

I, Paolo Pozzilli, declare as follows.

1. I am an Italian citizen residing in Rome, Via Fiera di Primiero 20, 00135.
2. I graduated in Medicine in 1976 from the University of Rome "La Sapienza" with top marks and honors.
3. I specialised in Endocrinology and Metabolic Diseases at the same University in 1979.
4. I was appointed Research Fellow in Diabetes in 1980 at St.Bartholomew's Hospital, London UK. At that time I received a fellowship from the Juvenile Diabetes Foundation (USA) to study the immunopathogenesis of Type 1 diabetes. Since then I dedicated all my research and clinical activities to the cause of diabetes and its complications.
5. In 1982 I was appointed Assistant Professor of Endocrinology at the University of Rome "La Sapienza".
6. In 1985 I moved back to London at St.Bartholomew's Hospital where I was given the title of Senior Lecturer in Medicine.
7. In 1990 I was called to the Dep.t of Endocrinology and Diabetes, II Clinica Medica, Policlinico Umberto I, Rome.
8. In 1997 I moved to the Department of Medicine, Endocrinology Section of the University Tor Vergata, Rome, where I was appointed as Associate Professor of Endocrinology.
9. In 1999 I was called by the University Campus Bio-Medico in Rome as Full Professor of Endocrinology and Director of the Department of Endocrinology and Diabetes at the same University.
10. My current position is: Director of the Department of Endocrinology and Diabetes at the University Campus Bio-Medico in Rome and Professor of Clinical Research at St. Bartholomew's Hospital in London.
11. I am main author and co-author of more than 200 scientific articles published in peer reviewed journals (Pubmed, NIH Library) where you can find them under "Pozzilli P" (a list is herewith enclosed for sake of information – Annex A). I am co-Editor of Diabetes Metabolism Research & Reviews and member of Editorial Board of: *"Journal of Endocrinological Investigation"*, *"Diabetes Metabolism & Nutrition"*, *"Il Diabete"*. I review regularly for major diabetes and endocrine journals such as *"Diabetes"*, *"Diabetes*

Care", "Eur J. Endocrinology", "Diabetologia", "J. Endocrinology". I am member of many Scientific committees and Boards (a list is herewith enclosed for sake of information – Annex B). In particular I am member of the scientific Board of the *Juvenile Diabetes Research Foundation* (USA) and I review grants in the field of Diabetes for several Government and Board Institutions such the *European Union*, the *European Association for the Study of Diabetes*, the *Dutch Diabetes Foundation* and *Diabetes* UK.

12. I have been invited to give main lectures at the annual *Congresses of the American Diabetes Association* and the *European Association for the Study of Diabetes* and guest Speaker to several International meetings.
13. My work in the field of Type 1 insulin dependent diabetes has always been focussed into mechanisms responsible for the destruction of pancreatic beta cells leading to the disease, since my first major publication in the journal The Lancet in 1979. Therefore the original observation related to the role of beta casein (the object of the present patent application) in Type 1 diabetes is the result of more than 15 years of studies in the area.
14. My deep knowledge in the field of Type 1 diabetes leads me to declare the following statements. Several papers (enclosed in abstract form to this reply) have indicated that cow's milk consumption is positively correlated with Type 1 diabetes incidence. Obviously this concept does not mean that cow's milk causes Type 1 diabetes, however it prompted a number of investigators throughout the world to study why and what factors contained in cow's milk might be diabetogenic. Cavallo et al (our group) was the first to report in a major peer-reviewed journal (The Lancet - 1996) that beta casein present in cow's milk was an important factor in Type 1 diabetes as 1) it was recognised as antigen by lymphocytes of Type 1 diabetic patients only; 2) it differed in aminoacid sequence from human beta casein and thus causes an immune response in the host; 3) cow's milk beta casein has structural similarities with beta cell GLUT2 glucose transporter in beta cells. In should be mentioned that antibodies to GLTU2 were reported in the past and <u>nobody</u> was able to explain the reason for this abnormal response in Type 1 diabetes. Our paper in the Lancet had a tremendous impact on the media and major newspapers in US and Europe reported the news. Before the paper was public, the patent on beta casein and prevention of Type 1 diabetes by means of it removal was filed. Other investigators then moved to this area of diabetes research, confirmed and, most importantly, extended my findings demonstrating that beta casein consumption and not other fractions of caseins were correlated with Type 1 diabetes incidence in the different countries (low incidence of Type 1 diabetes in Iceland =

consumption of milk with low content of beta casein; high incidence in Finland = consumption of milk with high quantity of beta casein).

The relevance of my studies and results on the role of beta casein in the causation of Type 1 diabetes is confirmed by the fact that two major trials for the prevention of Type 1 diabetes using a cow's milk hydrolysate at birth in susceptible individuals to Type 1 diabetes have been implemented.

The first one, called **PREVEFIN (Prevention trial Finalised Italian Network - I am the coordinator for the trial in Italy)** is carried out in Italy. In this clinical trial infants with high genetic risk of developing diabetes as possessing the HLA genotype DR3/DR4 receive in their diet a cow's milk hydrolysate where beta casein is not present. A control group where ordinary cow's milk is administered acts for comparison. The outcome of the study is represented by the generation 1-3 years later of specific antibodies to pancreatic beta cells, a well recognised immunological sign which indicates progression versus full blown disease. The trial is supported by major agencies in the country including the Ministry of Health and the Ministry of University & Technological Science.

The other trial, called **TRIGR (Trial Reduce Insulin dependent diabetes in Genetically at Risk -trial of prevention of insulin dependent diabetes in the genetically at risk subjects – I am the coordinator for continental Italy**) is a large world wide study involving 42 centers. It is the major trial in terms of financial support that has been ever implemented in the field of diabetes. It is sponsored by the National Institute of Health of USA, the Juvenile Diabetes Research Foundation (USA)and the European Union. The approach is similar to Prevefin in that infants (nearly 3,000) to mothers or fathers with a family history of Type 1 diabetes are randomised to a cow's milk hydrolysate deprived of beta casein or ordinary milk as control. The outcome is the same of Prevefin, i.e. generation of autoantibodies to beta cells as a sign of the disease progression.

These trials are direct consequence of my inventive concept and reinforce the scientific relevance of my findings: primary prevention of IDDM is possible and this must be considered a major breakthrough in human health.

15. To strengthen my assertion in the above point 14., herein attached (Annex C) the Examiner will find a demonstration that lymphocytes from Type 1 diabetics show reactivity to (≡recognize) beta casein which cross reacts with the beta cell antigen Glut 2.

In order to be more specific, I and my coworkers generated T cell lines from blood of 9 patients with Type 1 diabetes with different genetic makeup related to HLA-DQ or DR

genotypes known to confer high risk to the disease. Therefore we first designed a number of overlapping peptides(n = 18) encompassing the whole sequence of bovine beta casein and a peptide from Glut2 (i.e. the suggested cross-reactive beta cell antigen with the beta casein peptide of interest). The sequence of Glut2 we created is identical to the sequence in position 63-67 of beta casein (Pro-Gly-Pro-Ile-Pro – Identified in the instant application as SEQ ID N. 2) corresponding to one of the region of variation between bovine and human beta casein. By culturing lymphocytes from diabetic patients and the peptide sequences we were able to generate specific T cell lines to beta casein.

Beta casein T cell lines reacted to different sequences of the protein, however a higher frequency of T cell reactivity was observed towards the C-terminal portion of the protein (peptides B05-14 and B05-17 in 5/9 (55%) and 4/9 (44%) of T cells, respectively.

Various other sequences of beta casein show immune reactivity when T cell lines from diabetic patients are tested, a clear demonstration of the immunological concept of epitope spreading towards beta casein, thus reinforcing its role as strong antigen in Type 1 diabetes.

Most important, T cell lines reacted to the homologous peptide from Glut2. The enclosed figures showed the results of this study.

Based on these findings, we can conclude that: a) T cell lines specific to bovine beta casein can be isolated from peripheral blood of patients with Type 1 diabetes; b) these cell lines react with multiple and different sequences of beta casein, particularly towards the C-terminal portion; c) a cross reactivity between Glut2 and beta casein has been demonstrated. Such data is the definitive proof that our discovery upon which the patent is based is true. Beta casein is a major antigen in Type 1 diabetes and its elimination from cow's milk represents a fundamental step towards prevention of the autoimmune response leading to Type 1 diabetes.

The results of this study should be considered <u>confidential</u>, they will be in press in Clinical & Experimental Immunology in the near future.

Rome, 12 March, 2002

Professor Paolo POZZILLI